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SERINE SYNTHESIS AND  
THE PROVISION OF CYTOSOLIC REDUCING POTENTIAL IN  
THE ISOLATED PERFUSED RAT KIDNEY

By

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## ABSTRACT

An isolated perfused rat kidney preparation was designed, constructed, and tested for the evaluation of specific aspects of the metabolism of aspartate and other glucose precursors by the rat kidney.

The major pathway active for the metabolism of aspartate by the perfused kidney was identified through the use of aminooxyacetate, an inhibitor of aspartate aminotransferase (E.C. 2.6.1.1) and had-acidin, an inhibitor of adenylosuccinate synthetase (E.C. 6.3.4.4). This pathway was found to involve aspartate transamination and not the purine nucleotide cycle. Thus the major source of ammonia under these perfusion conditions is via glutamate dehydrogenase (E.C. 1.4.1.3.) activity.

Glucose production from aspartate and from glutamate by perfused kidneys was found to be associated with the synthesis of serine. Serine is an amino acid that is normally released by the kidneys in vivo. Production of serine by the perfused kidney had not been previously observed. The results of these experiments strongly suggest that its synthesis is via the "phosphorylated" pathway. This pathway involves 3-phosphoglycerate, phosphohydroxypyruvate, and phosphoserine as intermediates. This pathway had not been thought to be active in the kidney in vivo. These conclusions are based on data obtained with the use of various <sup>14</sup>C-labelled substrates, inhibitors of specific sites of renal metabolism, and substrates which are metabolized by the kidney via documented

pathways.

The provision of cytosolic reducing potential was investigated since glycerate and aspartate were found to be glucogenic in the kidney. These substrates are known to be metabolized to intermediates which are oxidized with respect to glucose. The pathways responsible for glucose production from these precursors dictate that a mechanism must be operative for the provision of cytosolic reducing potential, in the form of NADH, for glucose production that is independent of the pathway involving direct flux of carbon from precursor to glucose. Serine synthesis, when kidneys were perfused with a nitrogenous substrate, was identified as one such mechanism. "Pyruvate cycling" was identified as a system for the shuttling of intramitochondrially generated reducing potential to the cytosol. This cycle was investigated through the use of  $\alpha$ -cyanohydroxycinnamate, an inhibitor of mitochondrial pyruvate transport, and 3-mercaptopicolinate, an inhibitor of phosphoenolpyruvate carboxykinase (GTP) activity (PEPCK, E.C. 4.1.1.32). The results demonstrated pyruvate to be an intermediate of aspartate metabolism by perfused kidneys. Evidence was also obtained for the operation of a pathway for pyruvate formation from malate that is independent of PEPCK activity. The activities of the NAD- and NADP-linked 'malic' enzyme were estimated to assess the capacity of these enzymes in rat kidney to catalyze pyruvate formation. Only the NADP-linked 'malic' enzyme (E.C. 1.1.1.40) was found to be of sufficient activity to account for the estimated rate of pyruvate formation in these studies.

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